A clinical method for the study of in-vivo adhesiveness of teeth

PER-OLOF GLANTZ*
MALCOLM D. JENDRESEN *;**
ROBERT E. BAIER***

*Department of Prosthetic dentistry, Faculty of Odontology, University of Lund, Sweden

**Division of Biomaterial Science, Department of Restorative Dentistry, University of California, San Francisco, California, USA

***Calspan Corporation, Buffalo, New York, USA

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For the present study a clinical method was developed to allow the direct or photographic recording of contact angles on solid surfaces within the oral cavity. This method, which was tested on four subjects, allows for relatively accurate and precise recordings of contact angles to be taken under relavant clinical conditions and without disruption of the surfaces in their normal state.

Key-words: Surface properties; methodology; contact angles

Per-Olof Glantz, Tandläkarhögskolan, Carl Gustavs väg 34, 214 21 Malmö, Sweden

The surface chemical properties of teeth and restorative materials used in dental treatments influence the adhesion of dental plaque (5), the detailed composition of pellicles (10) and the manner of molecular packing in the pellicles (2).

Limited previous *in-vivo* studies related to intra-oral surface phenomena (1) required analysis using special instruments and tests conducted under laboratory conditions.Since biological systems may be sensitive to changes in the environment, it was considered valuable to develop an *in-vivo* method for studies of the surface chemical properties. The technique selected for characterizing these surface chemical properties employed contact angle measurements to derive the *in-vivo* critical surface tension. Zisman (12) demonstrated a useful direct relationship between the practical adhesiveness of a solid surface and its critical surface tension of wetting.

Two methods were developed to obtain direct contact angle data *in-vivo:* a direct measurement method and a photographic one.

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MATERIALS AND METODS

Four males between the ages of 25 and 28 years were chosen as test persons for the present study. The four males, who considered themselves to be in good general health, were found to have no marked periodontal disease or any carious teeth upon clinical dental examinations.

The direct measurement method was conducted using a modification of the optical equipment described in detail by Glantz (5). This consisted of, in part; a microscope tube with focusing device, an aluminium box with surface-coated mirrors which reflected and projected images of the surface to be studied onto a frosted glass plate, and a protractor graduated in whole degrees. These pieces of equipment were mounted on a heavy adjustable stand. For the illumination, a standard laboratory lamp was used.

Direct measurements of contact angles formed from selected test liquids placed on the labial surface of the anterior teeth of human subjects were then accomplished by placing the test person between the optical equipment and the light source. The test person was positioned horizontally on a flat table and the head was supported to prevent lateral movements. The selected highly purified test liquids (1) and their measured surface tensions are given in Table 1.

A plantinum wire, gauge 16, was used to deliver microvolumes of each test liquid to the labial surface of a maxillary or mandibular anterior tooth. Immediately prior to the application of a test liquid the tooth was positioned horizontally and broght into focus on the frosted glass screen of the optical system. The test persons kept their mouths closed until approximately 30 seconds before the application of each liquid to the tooth surface. The test liquids were placed on the tooth individually and in random sequence. Within a two hour period immediately prior to each experiment the test persons consumed no food or drink nor were any tooth cleaning procedures performed. After a micro-drop of the test liquid had been brought into contact with the surface of the tooth, a final focus of the microscope tube was made when necessary. As soon as the liquid had stopped spreading over the surface and reached a mechanical equilibrium, its angle of contact was recorded according to the technique described by Glantz (5). At each experiment 4 angles were recorded between the tooth surface and every non-spreading test liquid.

Table 1. *Surface tension (γ_{LV}) at 22°C of liquids used for the measurement of contact angles*

Liquid	γ_{LV} (dynes/cm)
1. Water	72.4
2. Glycerol	63.7
3. Formamide	58.5
4. Thiodiglycol	53.5
5. Methylene jodide	51.7
6. S-Tetrabromethane	49.8
7. Propylene Carbonate	41.8
8. Dicyclohexyl	32.7
9. Hexadecane	27.6
10. n-Tridecane	26.0
11. Decane	23.9

The wire was heated over a flame unitil red and allowed to cool for only a few seconds before selection and application of each micro-drop or addition of fresh liquid to such drops.

After each recording the test liquid was removed from the tooth surface with a piece of chemically clean cotton and the test person was asked to close his mouth until the next liquid was applied. The clinical recordings of contact angles were repeated four to five times for each test person with time intervals of two to seven days between each of the clinical sessions.

A second method was developed in order to photograph the liquid drops on the surface of the tooth in-vivo and then subsequently measure the contact angles formed.

The optical equipment described in the direct measurement method was re-

Fig. 1. Test person, camera, and fiber optic light in position in order to photograph across the labial surface of anterior tooth.

placed by a 35 mm SLR camera fitted with a series of lenses to provide a 4X image.

The camera was a single lens reflex 35 mm, Nikkormat FTN model fitted first with a 2X teleconverter and then with a Deesen lens. (Lester A. Dine Inc. New Hyde Park, N.Y. 11040). This lens also provides a 2:1 image. The Deesen lens has a fixed F:45 diaphragm for extreme depth of field and a strobe ringlight.

The test persons were positioned horizontally on a flat couch. A fiber optic light source, type Interlux 100 HL (Volpi AG, Urdorf, Zürich, Switzerland) was used to illuminate the tooth surface with bright, cool light. The camera, mounted on a heavy mobile support stand, was positioned on the right side of the test person. The camera was aligned so as to view across the surface of the tooth to be tested. This provided a view approximately parallel and planar tọ the interface between the labial surface of the tooth and the liquid micro-drop applied to the tooth (Fig. 1).

Following the placement of test liquid, the image of the liquid drop on the tooth surface was focused sharply in the camera by adjusting the distance to the subject through the use of a horizontal compound.

High speed color film (Kodak Ektachrome 400, Eastman Kodak Co, Rochester, N.Y.) was used with the ringlight flash activated.

The resultant 2x2 color slides were then viewed through an X-ray film enlarger/viewer. (Realist Inc. Photographic Products Division, Menomonee Falls, Wisconsin 53051, (USA). When the image of the liquid drop on the tooth surface was projected on the enlarger/viewer screen the contact angles was determined with the use of a protractor graduated in whole degrees. The protractor had a movable arm with double indices to provide for parallactic adjustment (5).

The placement of the liquids on the tooth surface and the management of the test persons was the same for both the direct measurement method and the photographic method.

From the contact angles obtained, the critical surface tensions according to Zisman (12), were calculated using the equations and graphic modifications described by Glantz (6). As the recorded contact angles of water probably were of the receding type because of the high amount of water present in pellicles, water contact angles were not used at the calculations of the critical surface tensions for polar test liquids. Neither were the data obtained for formamide because of its possible dissolving action on pellicle material.

Studies on some possible sources of error in the method

Some potential sources of error in the two methods described were taken under special consideration.

Mechanical equilibrium

A state of mechanical equilibrium must be reached and maintained in order to obtain the correct angle of contact. Therefore the test persons must be comfortable so as not to produce movements that would disturb the state of mechanical equilibrium. Repeated measurements at different as well as the same sessions showed no significant differences in the recorded angles between the same and different tooth surface and a given liquid on the same test person.

Hysteresis effect

Contact angles are often not definite quantites but differ according to whether the liquid is advancing over a surface or receding from a previously wetted surface. Therefore all readings were taken on areas of teeth not previously wetted by the same test liquid during a single session. As already mentioned, because of the high water content of pellicles it was, however, still likely that most or all of the recorded water contact angles were of the receding type.

Contamination

The available area of tooth surface was not large enough to allow application of a test liquid on discrete areas not previously used during a single session. Therefore applications of all the test liquids to the surface of the tooth were performed in random order. The sequence of liquid application was not observed to have any effect on the magnitudes of the contact angles recorded where the patients were allowed to close their mouths momentarily between applications of different liquids.

Photogrammetry

The possible inherent errors in the film material used were studied by comparing repeated measurements taken on the same image with those obtained on different images of the same micro-drop. No differences could be detected.

RESULTS

The results of the performed wetting studies on the surfaces of anterior teeth of the test persons are given in Table 2.

Fig. 2. presents the complete recordings for test person no. 1 of the relationshiips between the surface tensions (γ_{LV}) of the various test liquids and the cosines of their respected contact angles (θ), as well as the calculated critical surface tensions for the polar (γ_c^p) and non-polar (γ_c^d) test liquids (6).

The results given in Table 2 and Fig. 2 indicate that the anterior teeth of the tested persons had low energy surfaces with similar wetting properties.

In order to study in detail the possible significance of differences observed between the angles recorded at different sessions on the same test person and between different test persons, an analysis of variance was performed on the data given in Table 2. The program used was statistical package for the social sciences Sperry Univac 1100 exec. 8, Version H. September 1978. The results of these analyses showed that there were no significant differences between any of the critical surface tensions calculated from angle recordings obtained from the same or different test persons.

Between the angle data for the individual test liquids, between the different test persons, statistically significant differences were found for three of the eight liquids, liquid 4 ($p < 0.005$), liquid 5 ($p < 0.001$) and liquid 8 ($p < 0.05$).

DISCUSSION

The surface chemistry of teeth and selected dental materials has been studied by several investigators (2, 3, 4, 5, 6, 9, 11). Several of these studies have produced results indicating that enamel and dentine are normally covered by low energy material which may in fact originate from the tooth itself and also that metal-

Table 2. *Mean* in vivo-*contact angles* (Θ) *between certain liquids and human enamel of four different subjects, and calculated critical surface tensions for polar* (γ_c^p) *and non-polar* (γ_c^d) *test liquids*

	Water			Glycerol			Formamide		
Subject	n	Θ	S.D.	n	Θ	S.D.	n	Θ	S.D.
1	4	26.4°	1.98°	4	44.8	2.77°	4	26.8°	3.31°
2	4	27.8°	2.68°	4	41.5°	10.19°	4	21.6°	14.53°
3	4	20.5°	13.68°	4	49.3°	3.39°	4	30.8°	4.16°
4	5	26.8°	6.00°	5	43.0°	4.09°	5	32.9°	1.90°

	Thiodiglycol			γ_c^p (dynes/cm)		
Subject	n	Θ	S.D.	n γ_c^p	mean	S.D.
1	4	27.9°	1.16°	4	45.7	4.50
2	4	31.0°	2.21°	4	59.8	30.77
3	4	34.0°	2.28°	4	42.1	6.23
4	5	30.4°	1.25°	5	41.6	4.68

	Methyleneiodide			S-Tetrabromoethane			Propylene Carbonate		
Subject	n	Θ	S.D.	n	Θ	S.D.	n	Θ	S.D.
1	4	44.4°	2.95°	4	35.5°	5.18°	4	31.4°	5.89°
2	4	45.0°	1.08°	4	35.9°	4.94°	4	29.5°	2.26°
3	4	58.3°	3.56°	4	48.0°	2.15°	4	34.3°	2.16°
4	5	46.6°	6.16°	5	33.8°	12.08°	5	32.1°	1.46°

	Dicyclohexyl			Hexadecane			γ_c^d (dynes/cm)		
Subject	n	Θ	S.D.	n	Θ	S.D.	n	mean γ_c^d	S.D.
1	4	Spreading		–			4	32.3	1.76
2	4	3.4°	6.87°	4	Spreading		4	32.3	1.28
3	4	12.2°	8.22°	4	Spreading		4	30.6	2.03
4	5	Spreading		–			5	32.0	1.53

lic restorative dental materials have hydrophilic surfaces probably of high free energy levels. In addition, it has been known for quite some time that so-called acquired pellicles form on solid surfaces in the oral cavity by adsorption of salivary glycoproteins (8). Baier & Glantz (2) studied such oral films which had formed *in-vivo* on surface modified germanium prisms. Among other things they found that the pattern of packing of the molecules forming these oral films depends on which type of experimental surface is employed. On high energy surfaces, the adsorbed protein molecules had a comparatively close packing whilst on organic low energy surfaces they were in a looser, probably more native (i.e. solution-like) arrangement. Approximately the same amount of film materials was formed on both types of surfaces but thicker films formed on organic rather than on metallic surfaces. The results of the present study, which obtained data directly from the surface of the tooth, are in good agreement with the prior investigation.

Since dental plaque develops through secondary attachment of microorganisms to adsorbed pellicles, interesting, and the most relevant questions in this respect, are whether or not the adhesiveness of pellicles is determined by the surface chemical characteristics of the materials on top of which they form, and, of course, if such adhesiveness differs between and within individuals, as well as between different sex and age groups.

As has previously been discussed by Glantz (5) the only available non-destructive method for studies of the potential adhesiveness of biological material is the wettability-method described by Zisman (12). In this study such a method has been developed and tested for clinical use and it is expected to be useful for future studies, e.g. of the background mechanism of plaque formation.

Two variations of the basic contact angle method were tested, one involving direct measurements, the other utilizing photographic records which were measured at a time removed from the clinical session. When the results of these two variations were compared, no sign of systematic differences was detected. Since the direct measurement variation can be both mentally and physically tiring for the test person as well as for the investigator, and since it is essential to make measurements/recordings as quickly as possible after the liquids have reached a mechanical equilibrium on the tooth surface, the photographic variation was favored.

Any photographic method has minor inherent errors (7). However, by aligning the camera so as to use the centermost part of the lens for the object, these inherent errors of the lens and the film were minimized.

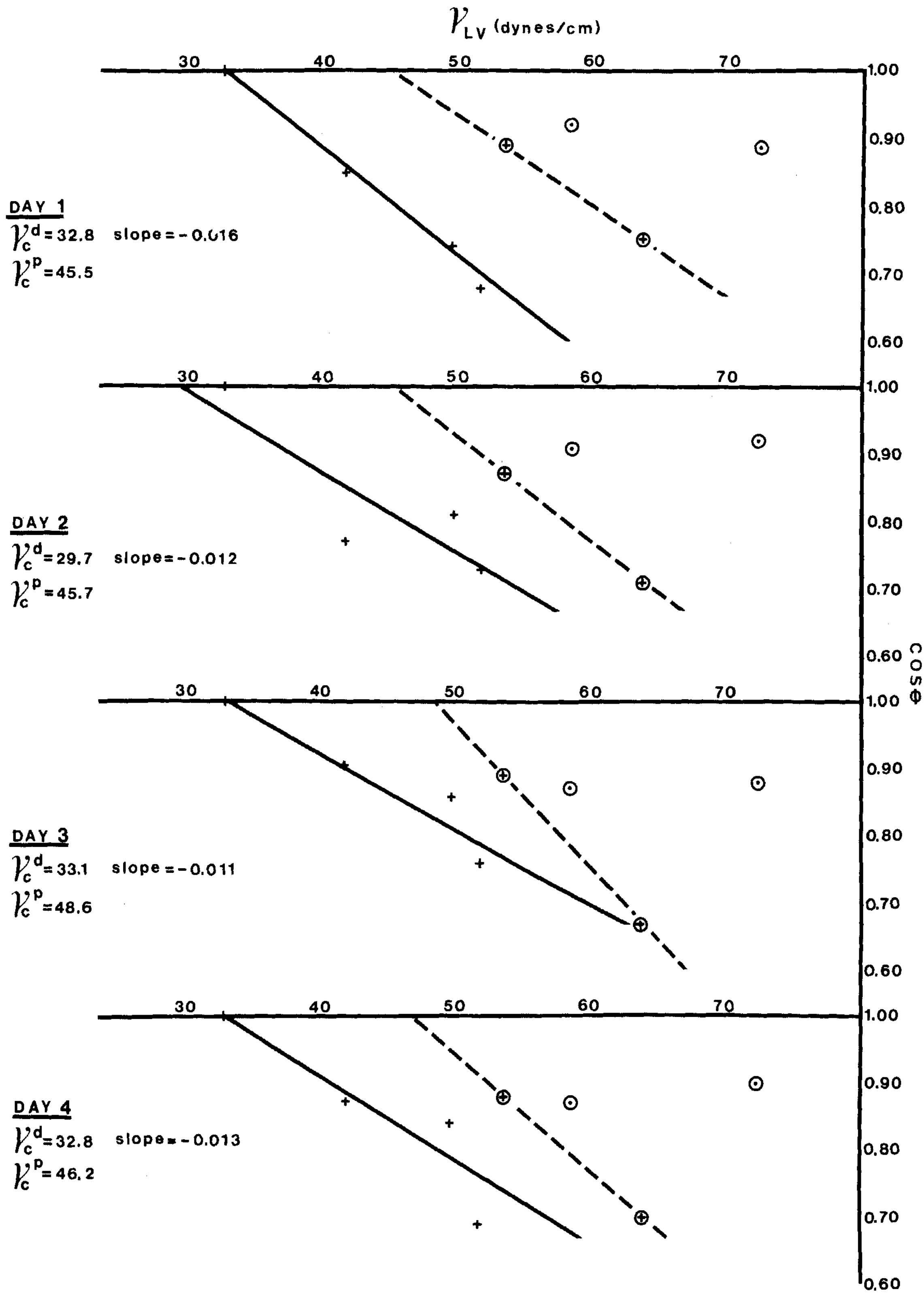
The photographic method has several advantages over the direct measurement technique. Photographs provide a permanent record of the test liquids interaction with the surface to be studied. This allows for repeated measurements of the contact angle if and when necessary. It further provides the convenience of measuring the angles at an alternate time to the clinical data collecting session. Finally the data collection is generally accomplished in a much shorter period of time with the camera. The camera is easier to focus than the much longer and heavier direct optical equipment described. The color film used in this technique provides a clear well defined image of the border between each test liquid and the surface being studied. This facilitates measuring the angles with the protractor.

The use of an enlarger/viewer is not necessary for determining the angles, which may be measured directly on the films or on a print. The print procedure includes, however, another generation image which could produce a minor distortion factor.

When the results of this study were examined the precision of the measurements was remarkably high, considering that recordings were made under complete clinical conditions. No major systematic differences were found in the performed studies of errors of the method, and this indicates that the described method is both valid and reliable. The clinical method of determining contact angles on solid surfaces and the resultant calculation of the critical surface tension of wetting of the tissue opens the door to a variety of future studies. For example, for the first time, a methodology exists for studying in situ the surface activity (the formation of orientated monolayers) in the presence of a disease process. These clinical methods may also serve to better characterize the surface chemical conditions in a caries prone mouth, or in a mouth prone to high plaque formation, as well as other conditions.

Further, the advantage of direct photographic clinical methods of recording contacts angles provides for the study of the undisturbed normal surface of the teeth and of dental restorative materials

Fig. 2. Relationship between the surface tension of certain test liquids (γ_{LV}) and the cosines of contact angles (cos Θ) recorded *in vivo* between these liquids and the labial surface of an anterior tooth, and calculated critical surface tensions (γ_c^p and γ_c^d), for one test person (1).



γ_{LV} (dynes/cm)
30
40
50
60
70
1.00
0.90
0.80
0.70
0.60
COS Φ
DAY 1
γ_c^d = 32.8 slope = -0.016
γ_c^p = 45.5
DAY 2
γ_c^d = 29.7 slope = -0.012
γ_c^p = 45.7
DAY 3
γ_c^d = 33.1 slope = -0.011
γ_c^p = 48.6
DAY 4
γ_c^d = 32.8 slope = -0.013
γ_c^p = 46.2

used for dental treatment. When the surfaces of teeth are studied in the laboratory, or when materials are removed from the oral cavity and later studied, the biological influence of the oral environment may be lost.

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